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Bionanotechnology New Frontiers

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ON THE COVER

"Bionanotechnology Research Potential" designed by Suresh Neethirajan and created by Christopher Stokes, Project Co-ordinator, University of Tennessee-Chattanooga.

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FEATURES

DEPARTMENTS

Bionanotechnology

New Frontiers

Suresh Neethirajan

Editor's Note: *With the help of staff scientists at the Oak Ridge National Laboratory, Suresh Neethirajan has developed novel microfluidic devices to sort bacteria, as well as image processing tools and nanoscale analytical techniques for studying systems biology, biofilms, and plant-microbe interactions. Neethirajan's discoveries will promote a more thorough understanding of biofilms and biomaterials.*

Bionanotechnology is essentially the study of biological ideas with nanotechnology. To put it in different terms, bionanotechnology is a miniaturized version of biotechnology, a field that centers on the use of living organisms and bioprocesses in engineering, technology, medicine, and other fields. Bionanotechnology is an emerging interdisciplinary field at the interface of biotechnology and nanotechnology. Because it's still in the early stages, the topical areas of bionanotechnology research cover a wide range. In particular, bionanotechnology is ideally suited for understanding the interfaces between organisms in systems biology.

To help understand bionanotechnology, it is important to know what biofilms are. Biofilms are organized structures, primarily made of exopolysaccharides, water, and microbes, that are formed by one or several species of bacteria attached to solid surfaces. Biofilms affect many aspects of human life, including industry, medicine, and biosystems. In particular, biofilms play a major role in plant-microbe interactions, biofouling, and biocorrosion.

The challenges associated with characterizing the diverse organisms involved in plant-microbe interfaces and dissecting their molecular exchanges are being addressed in collaborative research with my fellow scientists at Oak Ridge National Laboratory. Together, we have developed a variety of analytical tools for use in bionanotechnology, including microfluidic devices to sort bacteria, as well as image processing tools and nanoscale analytical techniques.

Microfluidics

Microfluidics is the science of constructing tiny (or microminiaturized) devices with tunnels and chambers for the precise control and manipulation of fluids. Within the 10 to 100 micron channels of these devices, fluid flow is dominated by surface tension and laminar effects. Since biofilm processes are initiated in confined microscopic spaces, and the size of bacterial cells is on the micron scale, microfluidic systems provide major advantages in studying biofilms. In particular, these systems have unique capabilities for applying stimuli to individual cells or to groups of cells and observing the responses.

Unlike conventional benchtop systems, microfluidic systems allow efficient control of concentration gradients and avoid mechanical stresses in characterizing biofilms. For example, microfluidic devices have been designed and fabricated to study the influences of hydrodynamics in the bacterial environment, which helps us understand biofilm formation and adhesion kinetics. Combined with nanoscale features, microfluidic devices also aid in functionally assaying bacteria to investigate species variation.

Our work in microfluidics has been innovative, and it has provided some new developments. For example, we have developed microfluidic methods to sort bacteria based on their affinity to chemo attractants. Using PDMS (polydimethyl siloxane) material, microchannels were created on a glass surface. The nanoporous barriers between the fluidic channels confined the bacteria in distinct compartments while allowing controlled delivery and exchange of molecular cues between the compartments (fig. 1).

An understanding of the complex chemical communications between plants and the bacteria that surround the plant roots was also achieved using microfluidic systems. The bacteria are attracted by chemicals that are produced by the plant roots and travel toward them for biofilm formation. Sometimes, for various reasons, the bacteria form colonies on the root surface. Without microfluidic systems, it would not be possible to understand the behavior of these bacteria.

In addition, fabricated nanostructured microfluidic devices have helped us understand the effect of chemical cues on cellular processes, such as surface recognition, adhesion kinetics, cell-

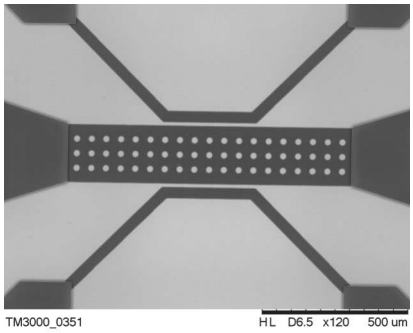

Figure 1. SEM image of a nanopore microfluidic channel for microbial isolation and characterization.

cell communication, and chemotaxis. Microfluidic devices are small and portable, which makes them suitable for screening field isolates of bacteria as well as for food quality monitoring. The devices can also be used to emulate drug delivery using tissues cultured inside the microfluidic compartments.

Image processing and nanoscale analysis

Imaging studies of the colonization and surface adhesion kinetics of bacteria using atomic force microscopy (AFM) and confocal laser scanning can reveal the evolution of microbial biofilm morphologies and the structure of the bacterial pili (the hair-like appendages found on many bacteria). By understanding the way bacteria attach to different surfaces, it is possible to create nano-patterned surfaces so that biofouling and biocorrosion activity can be minimized or avoided.

Quantitative imaging of live biological samples has also been achieved using AFM and confocal laser scanning. We quantified the pico-force with which the bacteria (fig. 2) are attached to different surfaces using mica, polystyrene, polypropylene, and glass substrates. Non-intrusive investigation of single biomolecules is possible, and it is useful for screening and diagnostic purposes, as there are connections between biomarkers and genetic disorders.

We have also been successful in producing nanowires from bacteria. These nanowires are 6 to 8 nm in diameter (fig. 3). They can conduct electrons and could be used as single-molecule electronic devices. To produce them, the growth conditions of bacteria were optimized to make the bacteria express nanowires from specific field isolates. The nanowires were then deposited on mica substrates and imaged using atomic force microscopy and transmission electron microscopy. In the near future, we will be able to grow nanowires in quantity, and researchers will create biological circuits with this new micromaterial.

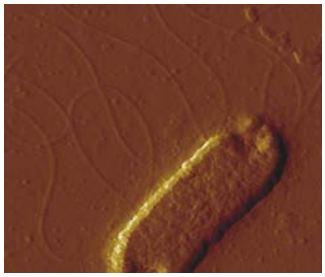

Figure 2. Atomic force microscopy image of *Populus* root bacteria YR505 with multiple polar flagellum.

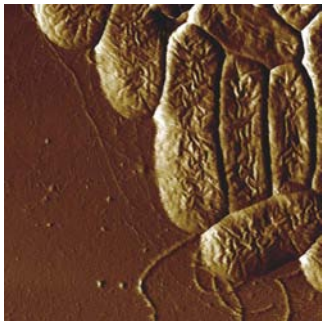

Figure 3. Bacterial nanowires produced from the field isolate YR505 of poplar tree root rhizosphere.

The association of plants and microbes can often benefit plant health. Often, though, little is known about the specific organisms involved or the mechanisms through which these processes occur, particularly in natural ecosystems. Therefore, we examined the spatial and temporal dynamics of microbial colonization of *Populus* roots using microbial isolates that express green fluorescent protein (GFP). The association and attachment of bacteria to *Populus* roots is an initial step in microbial colonization and is influenced by numerous factors, including molecular signaling events, bacterial transport, and surface recognition. Standard molecular methods were used to introduce GFP into microbial isolates. Confocal and atomic force microscopy were then used to characterize the morphology, surface characteristics, and dynamics of biofilm formation of selected microbes isolated from the *Populus* rhizosphere.

The gamma-proteobacteria isolate YR343 was observed to attach to the *Populus* roots (fig. 4) after approximately five hours of co-culture. The cells were observed to grow and form colonies on the surface of the root. Expression of pili during the biofilm formation and distinct morphotypes were revealed by the image analysis. These colonization studies provide direct evidence that microbes collected from the rhizosphere associate directly with *Populus* roots, and the dynamics of colonization were observed in real time using GFP-expressing microbes. The methods developed for this study will enable additional studies aimed at investigating plant and microbial responses to colonization.

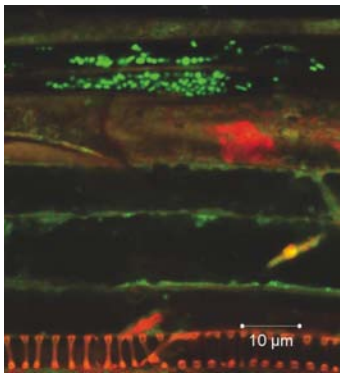

Figure 4. Confocal laser scanning microscopy image of YR343 biofilm colonization along the plant cell wall.

To sum it up

Our research in the field of bionanotechnology has been highly productive. We have developed novel image processing tools and nanoscale analytical techniques to study systems biology, biofilms, and plant-microbe interactions, and we have designed and fabricated microfluidic devices and systems for studying bacterial chemotaxis. In particular, we have been highly successful in studying how bacteria attach to root surfaces and in imaging live bacteria colonization on root surfaces. Last but not least, we have developed tools to create DNA templates and analyze chromosome structures using atomic force microscopy. All that, and the field of bionanotechnology is just beginning.

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Access the lab's web page at www.bionanolab.ca.